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Carbamate-Resistance in Mosquitos

Selection of *Culex pipiens fatigans* Wiedemann (= *C. quinquefasciatus* Say) for Resistance to Baygon*

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The authors investigated the rate of development and other characteristics of Baygon-resistance in Culex pipiens fatigans (=C. quinquefasciatus) *recently colonized from Southern California. Selective pressure against the larvae (strain L) for 35 generations resulted in 25.4-fold resistance in larvae, but only 3-fold and 4.8-fold resistance in adults, as determined by contact and topical application, respectively. Conversely, selective pressure on adults (strain A) for 24 generations resulted in 8.4-fold (contact) and 5.3-fold (topical) resistance in adults, but only 2.7-fold resistance in larvae. About 10% of strain A adults survived 1-hour contact exposure even when the concentration of Baygon was increased 100-fold. This was not due to enhanced phototropism in this stage. Larvae of strain L metabolized ^{14}C-labelled Baygon at a rate 2.5-fold greater than the non-selected strain. Cross-resistance in strain L to carbamates closely related to Baygon was high, but was extremely low to remotely related carbamates.*

The authors consider the relatively slow rate of development of resistance to Baygon, the reduced expressivity of the resistance character in adults, and its specificity for the selective agent and for closely related carbamates to be encouraging indications as to the usefulness of this class of compounds for mosquito control.

The development of resistance to organochlorine insecticides by anopheline and culicine mosquitos has created serious repercussions in mosquito control and eradication campaigns. The problem has been reviewed extensively in published papers (see Georghiou, 1965c), in seminars [4] as well as in several recent unpublished communications to WHO. As organophosphates were introduced to replace organochlorine insecticides, cases of resistance to these also appeared, particularly in species of *Culex* and *Aedes*. The development of resistance to malathion and diazinon in *Culex fatigans* in the Cameroons (Hamon & Sales, 1963) and Sierra Leone (Thomas [5]), to malathion in *Culex tarsalis* in California (Lewallen, 1961) and to diazinon, malathion and trichlorfon in *Aedes aegypti* in Puerto Rico (Fox & Garcia-Moll, 1961; Kerr et al., 1964) has provided ample evidence that the problem of resistance continues to exist despite the change to organophosphate insecticides. Of particular concern has been the phenomenal organophosphorus-resistance in larvae of *Aedes nigromaculis* (in California), which was estimated to have reached a level 4000-fold greater than initially to parathion, 20-fold to

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[4] *Bull. Wld Hlth Org.*, 1963; and papers presented at the WHO seminars on the ecology, biology and control of *Culex pipiens* and *Aedes aegypti* (*Bull. Wld Hlth Org.*, in press).

[5] Cited by J. Mouchet in an unpublished communication to WHO.

methyl parathion and fenthion, and 10-fold to malathion (Brown et al., 1963). Fortunately, no organophosphorus-resistance has yet been reported in field or laboratory populations of anopheline species. This, however, cannot be considered evidence of the absence of organophosphorus-resistance genes in these mosquitos. The phenomenon may well be due to lack of specific selection for such resistance. Thus, continued interest in insecticide-resistance—including research on the mechanisms of resistance, development of new, effective insecticides and the determination of the resistance potential of mosquitos to these—is of profound importance to sustained mosquito control. Answers to these problems are all the more important in view of the role of many mosquito species in the transmission of a multitude of human diseases and of the vast expenditures and efforts already invested in current vector control or eradication programmes.

As a result of extensive research into new insecticides, considerable interest has been generated during the last decade in the insecticidal properties of carbamic acid esters, largely with the expectation that organophosphorus-resistant strains of insects might be controlled effectively by these compounds. Although this has not proven generally true with respect to houseflies (Georghiou, 1962), the high insecticidal properties of many carbamates justify their thorough exploration for mosquito control.

A comparative study of various carbamate, organophosphorus and organochlorine insecticides (Georghiou & Metcalf, 1961b) revealed that, while most of the carbamates studied are relatively weak larvicides, they possess outstanding toxicity to adult mosquitos. In that study, only two compounds, trichlorfon and lindane, which are known to possess strong fumigant action, showed adulticidal activity greater than that of the more toxic carbamates (compounds II, VII and X in Table 1). This discovery, as well as a report on the residual activity of compound II (LaBrecque et al., 1960), marked the beginning of a constantly increasing body of information on various aspects of carbamate toxicity to mosquitos. Of some 1170 chemicals submitted to our laboratory during 1961-65 by various commercial or public concerns for evaluation under the WHO-sponsored Insecticide Evaluation Programme, about 17% were carbamates. Many of these have been discussed in various papers dealing with comparative toxicity (Georghiou & Metcalf, 1961b; Metcalf et al., 1962, 1963; Metcalf & Fukuto, 1965; Hadaway & Barlow, 1963, 1965a, 1965b); with behaviour on treated surfaces (LaBrecque et al., 1960; Gahan et al., 1961a, 1961b, 1962, 1964; Schoof et al., 1964; Bar-Zeev et al.;[1] Hadaway & Barlow, 1965a, 1965b); and with field performance (Vandekar, 1965;[2] Bar-Zeev & Bracha, 1965). The use of carbaryl (compound XXIII in Table 1) in a village trial in Southern Nigeria was considered unsuccessful owing to its short persistence on mud walls (Bar-Zeev & Bracha, 1965), but trials with this material and compound XIX in whitewashed mud huts in Haiti gave satisfactory kills for 21 weeks (Schoof et al., 1964). Clinical observations during a trial with Baygon (III) in Southern Nigeria did not reveal any adverse effects either on spraymen or on exposed villagers.[2] A question of profound importance at this stage is the potentiality of mosquitos to develop resistance to carbamates and the character of such resistance.

Since 1961, we have been investigating various aspects of resistance to carbamates in mosquitos. Strains of *Culex pipiens fatigans* Wiedemann (=*C.p. quinquefasciatus* Say) and *Anopheles albimanus* were subjected to rigorous selection pressure in the larval stage with Hercules 5727 (compound II) but these selections were discontinued after 50 and 21 generations, respectively, since the tolerance levels to the carbamate did not rise above 2-fold and 2.7-fold, respectively (Georghiou, 1963; Georghiou & Metcalf, 1963). Nearly similar results with this compound on *Culex fatigans* are also reported by Brown & Tadano (to be published). Although very encouraging, these results are only of academic interest since Hercules 5727 appears to offer no possibilities for field application owing to its high mammalian toxicity (rat oral LD_{50}=12 mg/kg). Therefore, our attention was turned to Baygon (compound III) (rat oral LD_{50}=175-200 mg/kg), and an intensive study of resistance was initiated with the use of recently colonized strains of the above species. Interim reports on these selections have been given (Georghiou, 1965b; Georghiou & Gidden[3]). This paper and others to follow will include comprehensive accounts of these investigations, dealing with the results of carbamate selection, and with the toxicological, physiological and genetical characterization of the selected strains.

[1] Unpublished document WHO/Vector Control/167.65.
[2] Unpublished document WHO/Vector Control/177.65.
[3] Unpublished document WHO/Vector Control/187.65.

TABLE 1
IDENTITY OF CARBAMATE COMPOUNDS STUDIED

Compound No.	WHO identification No.	Chemical name	Source and/or manufacturer's designation
I	OMS-32	*o*-isopropylphenyl methylcarbamate	Bayer 39731
II	OMS-15	*m*-isopropylphenyl methylcarbamate	Hercules 5727
III	OMS-33	*o*-isopropoxyphenyl methylcarbamate	Baygon (Bayer 39007)
IV	OMS-436	*o*-propargyloxyphenyl methylcarbamate	Hercules 9699
V	OMS-177	*m*-propargyloxyphenyl methylcarbamate	Hercules 8717
VI	OMS-313	*o*-*sec*-butylphenyl methylcarbamate	Bayer 41637
VII	OMS-30	*m*-*sec*-butylphenyl methylcarbamate	Chevron RE-5305
VIII	—	*o*-*sec*-butoxyphenyl methylcarbamate	R. L. Metcalf
IX	—	*m*-*sec*-butoxyphenyl methylcarbamate	R. L. Metcalf
X	OMS-31	*m*-*tert*-butylphenyl methylcarbamate	Chevron RE-5030
XI	OMS-227	*m*-*sec*-amylphenyl methylcarbamate	Chevron RE-5353
XII	OMS-1028	*o*-cyclopentylphenyl methylcarbamate	Bayer 38799
XIII	OMS-1029	*o*-cyclopentenylphenyl methylcarbamate	Bayer 38800
XIV	OMS-864	2,3-dihydro-2,2-dimethylbenzofuranyl-7-methylcarbamate	Niagara 10242
XV	OMS-716	3-isopropyl-5-methylphenyl methylcarbamate	Schering 34615
XVI	OMS-22	2-chloro-3-isopropylphenyl methylcarbamate	Hercules 7522
XVII	OMS-575	3,5-di-isopropylphenyl methylcarbamate	Hooker HRS-1422
XVIII	OMS-597	3,4,5-trimethylphenyl methylcarbamate	Shell SD-8530
XIX	OMS-93	4-methylthio-3,5-xylyl methylcarbamate	Bayer 37344
XX	OMS-47	4-dimethylamino 3,5-xylyl methylcarbamate	Zectran (Dowco 139)
XXI	OMS-174	6-chloro-3,4-xylyl methylcarbamate	Upjohn 12927
XXII	OMS-1059	*m*-*sec*-butylphenyl-*N*-methyl-*N*-(methylthioacetyl) carbamate	Boots RD 17955
XXIII	OMS-29	1-naphthyl methylcarbamate	Carbaryl (Union Carbide)
XXIV	OMS-744	2-(methylcarbamoyloximino)-1,3-dithiolane	American Cyanamide 38906
XXV	OMS-764	2-(methylcarbamoyloximino)-4-methyl-1,3-oxathiolane	American Cyanamide 47548
XXVI	OMS-771	2-methyl-2-(methylthio) propionaldehyde-*O*-(methylcarbamoyl) oxime	Temik (Union Carbide 21149)

MATERIALS AND METHODS

Strains employed

The stock strain utilized in these selections was collected in 1963 in the Coachella Valley of Southern California and is referred to as the " Coachella " strain. The area is typically an irrigated desert where mosquito breeding takes place almost exclusively in irrigation canals and catch basins. The mosquito population is exposed to a variety of organochlorine and organophosphorus insecticides employed on the many different crops grown in the valley, and, in addition, it is subjected to direct chemical control by the Coachella Mosquito Abatement District. Detailed data presented later in this paper indicate that the Coachella Valley population sampled in 1963 was moderately resistant to DDT and it also exhibited low levels of tolerance to a number of organophosphorus insecticides and dieldrin. Reference to the susceptibility of this population to various insecticides has also been made by Mulla (1964).

A second strain of this species, collected in the San Joaquin Valley of California in 1950, has served

as the susceptible reference strain for comparison with the Coachella and carbamate-selected strains. This strain, designated " Laboratory ", has been under continuous rearing since its colonization, without being intentionally exposed to insecticides.

Rearing method

The adult colonies are maintained in cages measuring 46 cm × 46 cm × 46 cm with 18-mesh screen at the back and top. A sleeved opening, 15 cm (7 openings per linear cm) in diameter, provides access to the cage. A smaller screened cage in the interior, measuring 20 cm × 20 cm × 20 cm serves to house up to five baby chicks which provide the colonies with blood meals. The chicks are kept continuously in the cage for one week and are then replaced by new one-day-old chicks. They have access to food and water provided in suitable containers attached to the outside of the colony cage. Several hundred mosquitos can be maintained in each cage. Each colony is provisioned with crushed raisins and a waxed paper cup containing cotton saturated with 10% sucrose solution for adult nourishment. A second cup with water serves as an oviposition container. Egg rafts are collected daily and transferred to enamelled pans for larval development. In the second instar, the larvae are thinned to a density of about one per 4 ml of water. Larval food consists of finely ground Purina Laboratory Chow and hydrolized yeast in equal proportions. The larvae obtained under these conditions are uniformly large and vigorous. The pupae are either returned to the colony cage or are placed in a 1-US-pint (0.47-litre) waxed paper cup inside a 1-US-gallon (3.8-litre) ice-cream carton for adult emergence, for use in toxicological or other tests. These containers are equipped with a cloth sleeve at one end and an 18-mesh screen at the other, and have been found very suitable for maintaining adult populations pending their use in tests. The cup holding the pupae is transferred daily to clean cages so that the age of emerging adults is known within ± 12 hours.

Biological differences between the strains

The Coachella and Laboratory strains were reciprocally interfertile. It should be noted in this connexion that extensive tests by Barr [1] have revealed no cytoplasmic incompatibility among populations of this species from different geographical areas of the USA, although some strains were found to be incompatible with certain foreign strains. The only obvious difference between the Coachella and Laboratory strains was the slower developmental rate of the former as shown below:

	Days from egg deposition to pupation	
	Laboratory	*Coachella*
First pupa	11	11
25% pupation	14	15.3
50% pupation	15.3	17.8
75% pupation	17.6	20
100% pupation	21	25

Also obvious was a longer pre-oviposition period in the Coachella strain which was not determined quantitatively. These differences may be due to unintentional selection of the Laboratory population for faster development during the 15 years since its colonization.

Insecticide-testing techniques

All toxicological tests were aimed at establishing complete dosage-mortality regression lines (ld-p lines) by utilizing five or more dosages of the respective insecticides within the range producing 5% to 95% mortality. Each test was replicated five or more times at each concentration level, with populations reared on different days. The larval testing procedure is essentially the same as the standard recommended by the WHO Expert Committee on Insecticides (1963) with certain modifications which were found desirable in our laboratory. Briefly, early fourth-instar larvae are tested in groups of 20 in waxed paper cups (Dixie cup 346, 6 US fluid ounces; ca 180 ml) containing 100 ml of water. The insecticides are prepared in standard w/v acetone solutions of the desired concentrations and are applied at the rate of 1 ml per 100 ml of water. The results are assayed 24 hours later.

The adult testing method as already described (Georghiou & Metcalf, 1961c) consists of evaporating 1 ml of standard w/v acetone solutions on 9-cm Whatman No. 1 filter-paper, rolling the latter inside a 2.1 cm × 8.4 cm vial, and exposing 20 three-day-old sugar-fed mosquitos of mixed sexes to it. After one hour they are transferred to unwaxed paper cups (Dixie cup 2168) that are fitted with snap-on, clear plastic lids; inside each cup is placed a piece of dental roll moistened in 10% sucrose solution. The results are recorded 24 hours later. This method was modified during the later stages of

[1] Barr, A. R. (1965) *Cytoplasmic incompatibility.* In: *University of California . State Department of Public Health Mosquito Project. Fourth Quarter 1965* (Unpublished document) pp. 6-11.

this work by the substitution of glass-fibre filter-paper (Whatman GF/A 9 cm) for cellulose paper, since the former was found to give more consistent results, especially with carbamate insecticides (Georghiou & Gidden, 1965).

In a limited number of tests a topical application method was used. This was similar to the one described for houseflies by March & Metcalf (1949) and involved the application of 0.3 μl of acetone solutions of insecticides to the nota of three-day-old mosquitos held briefly under CO_2 anaesthesia.

Insecticides

All insecticides used were of the highest obtainable purity or were purified further by crystallization.[1] Acetone solutions of these compounds were kept either in a refrigerator at 1°C-2°C or in a freezer at −15°C. In either case, carbamate solutions were discarded every third month and remade when necessary, since it was found that the activity of certain compounds in this class diminished with long storage.

Selection techniques

Two strains were developed from the Coachella strain by Baygon selection pressure: strain L was selected by larval pressure and strain A by adult pressure. The larval selection technique consisted in exposing groups of 50 fourth-instar larvae in 100-ml of water in waxed paper cups to the required concentration of insecticide delivered in 1 ml of acetone. Exposure was for 24 hours, at the end of which the survivors were rinsed in clean water and placed in a rearing pan for pupation. Some 5000 larvae were treated per generation at a selection level of 80%-95% mortality. Selection of strain L began in June 1963, and by October 1965 altogether 35 generations were completed under pressure.

The adult selection technique, originally developed in this laboratory for selection of houseflies,[2] consisted in immersing the mosquitos (when <24 hours old) in an emulsion of Baygon as follows. 1 ml of acetone solution of the required concentration of the insecticide and one drop of an emulsifier (Triton X-100) were placed in 100 ml of water in an Erlenmeyer flask. The liquid was stirred gently so that no foam was formed on the surface. Anaesthetized mosquitos were dropped into the flask, which was shaken gently for about 10 seconds to ensure that the mosquitos were below the surface and thoroughly wetted. The water and mosquitos were then poured on to filter-paper in a Buchner funnel and dried in a gentle stream of air until the paper could be removed intact. The mosquitos were then transferred to paper cups, as described earlier, and the results evaluated 24 hours later. Beginning with the fourteenth generation of strain A, the selection technique was changed to one-hour contact exposure on filter-paper as described above under "Insecticide-testing techniques". A uniform interval of two hours was observed between treatment of the filter-paper and exposure of the insects to it. Each paper was used twice at the most, on two consecutive days. Of 27 generations completed by strain A, 12 were selected by dipping, 11 by contact and 4 were left unselected in order to avoid extreme reduction of the population. The selection pressure as in the case of strain L ranged from 80% to 95% mortality.

Treatment of data

The data, whether pertaining to changes in the susceptibility of the strains to the selective agent or to changes in their cross-resistance, were subjected to probit analysis by the method of Finney (1952) on a programmed IBM 7040 computer.[3] It was thus possible to detect when statistically significant changes in the susceptibility levels of different generations to various compounds occurred during selection.

RESULTS AND DISCUSSION

Development of resistance by larval pressure in strain L

The progressive changes in susceptibility to Baygon in larvae of strain L are indicated in Table 2. Complete ld-p lines are given in Fig. 1. It will be noted that the susceptibility of the parental (Coachella) and Laboratory strains to Baygon was about equal at the LC_{50} level, but the slope of the ld-p line of the Coachella strain was lower (b=3.3 *versus* 5.3), a phenomenon characteristic of field populations. A sharp increase in tolerance to Baygon in the F_1 generation occurred (4.4-fold) but progress thereafter was very gradual, eventually attaining a 25.4-fold level in the F_{35} generation. The slope (b) of the ld-p lines varied somewhat in the several generations examined, changing from an initial value of 3.3 in

[1] The authors are deeply grateful to Dr T. R. Fukuto for assistance in this connexion.

[2] March, R. B. & Printy, G. E. (1964) In: WHO Information Circular on Insecticide Resistance No. 43 (item 12).

[3] The authors appreciate the financial assistance of the Computer Research Fund of the University of California, Riverside, Calif., for the analysis of the data.

TABLE 2

CHANGES IN SUSCEPTIBILITY TO BAYGON IN LARVAE OF *C. FATIGANS* DURING SELECTION WITH BAYGON

Strain and generation tested	Baygon LC_{50} (ppm)	95% Fiducial limits	Slope (*b*) ±SE (*b*)	RR [a]
Laboratory	0.228	0.219-0.237	5.274±0.253	1.0
Coachella (P)	0.257	0.236-0.278	3.287±0.195	1.1
Baygon L				
F_1	1.008	0.956-1.064	5.397±0.357	4.4
F_7	1.598	1.356-1.794	10.679±1.834	7.0
F_{10}	2.001	1.949-2.053	8.427±0.532	8.8
F_{13}	2.213	2.071-2.386	6.538±0.607	9.7
F_{15}	2.787	2.704-2.872	6.893±0.429	12.2
F_{17}	3.118	3.056-3.180	9.037±0.379	13.7
F_{24}	3.054	2.813-3.294	7.843±0.922	13.4
F_{30}	4.193	3.818-4.648	8.178±1.207	18.4
F_{35}	5.785	5.580-5.997	6.900±0.418	25.4
Baygon A				
F_{24}	0.625	0.594-0.658	3.768±0.189	2.7

[a] RR (resistance ratio) = LD_{50} selected strain ÷ LD_{50} Laboratory strain.

the P generation to a high 10.7 in the F_7 and dropping to 6.9 in the F_{35}. These changes may reflect reorganization of genetic factors contributing to resistance. The maintenance of high slope values in all generations is in striking contrast to the situation characteristic of chlorinated-hydrocarbon resistance, where the ld-p lines exhibit a marked reduction in slope values during selection.

In contrast to the relatively high resistance to Baygon in larvae, the adult mosquitos of strain L exhibited only small increases in tolerance to this material. Contact and topical application tests on F_{35} adults indicated 3-fold and 4.8-fold increases in tolerance, respectively (see Table 4 and Fig. 3). Adult susceptibility is discussed later in connexion with strain A.

Stability of resistance in strain L. In order to test the stability of resistance in strain L, several hundred larvae of generations F_9, F_{22}, and F_{24} were reared separately without insecticidal pressure for a variable number of generations and the ensuing changes in their susceptibility were determined. The data in Table 3 indicate that when selection was suspended in the F_9, only a small regression in resistance occurred during the ensuing 10 generations, i.e., from a 9.46-fold level in the F_9 to a 6.6-fold level in the F_9 ($+F_{10}$ unselected). This might suggest

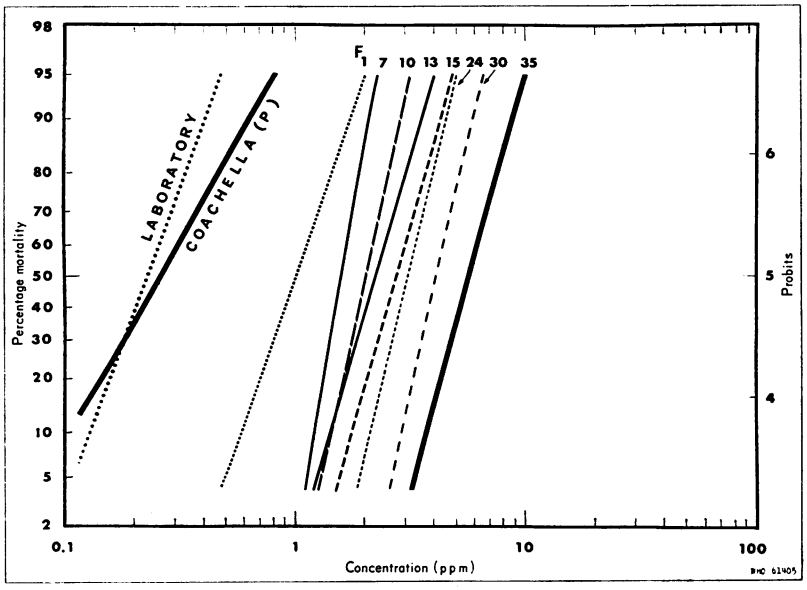


FIG. 1

LD-P LINES FOR VARIOUS GENERATIONS OF *C. FATIGANS* (STRAIN L) SELECTED BY BAYGON PRESSURE IN LARVAL STAGE

TABLE 3

STABILITY OF RESISTANCE TO BAYGON IN GENERATIONS F_9, F_{22}, F_{24} AND F_{42} OF STRAIN L REARED WITHOUT FURTHER INSECTICIDAL PRESSURE

Last selected generation	Generations without selection	Baygon LC_{50} (ppm)	95 % Fiducial limits	Slope (b) ± SE (b)	RR [a]
F_9		2.156	2.099-2.215	8.609±0.516	9.5
	F_1	2.005	1.946-2.066	8.795±0.577	8.8
	F_2	1.929	1.859-2.001	7.186±0.464	8.5
	F_4	1.933	1.886-1.980	8.179±0.429	8.5
	F_6	1.597	1.544-1.652	7.636±0.491	7.0
	F_{10}	1.504	1.404-1.603	8.464±0.800	6.6
F_{22}		2.459	2.401-2.518	8.301±0.477	10.8
	F_1	0.656	0.459-0.781	6.090±0.956	2.9
F_{24}		3.054	2.812-3.294	7.843±0.922	13.4
	F_1	1.870	1.731-2.011	4.880±0.425	8.2
	F_2	1.324	1.268-1.382	4.971±0.284	5.8
	F_4	0.358	0.336-0.380	3.760±0.277	1.7
	F_6	0.319	0.261-0.388	2.157±0.299	1.4
F_{42} [b]		5.048	4.915-5.185	7.837±0.464	22.1
	F_2	5.528	5.077-6.268	6.942±1.055	24.2
	F_3	3.949	3.664-4.215	8.849±0.874	17.3
	F_6	2.010	1.884-2.144	2.902±0.157	8.8
	F_7	2.317	2.217-2.421	3.942±0.167	10.2
	F_{10}	1.353	1.218-1.494	4.126±0.388	5.9

[a] RR (resistance ratio) = LC_{50} generation studied ÷ LC_{50} Laboratory strain.

[b] Since completing this manuscript, we have had the opportunity to re-examine the question of stability of resistance to Baygon by using generation F_{42} of strain L. The data obtained are essentially similar to the earlier ones and have been added to this table without additional comment in the text.

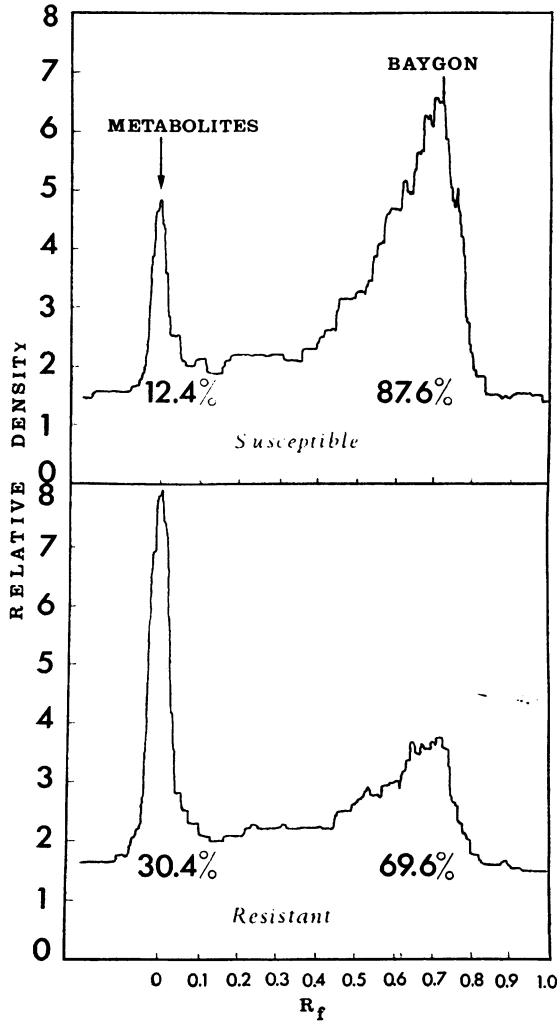
that the respective genes for resistance had been fixed in the population so that only a small loss in resistance occurred when selection was suspended. Abedi & Brown (1960) have emphasized the role of ancillary genes in the stability of DDT-resistance in *Aedes aegypti*.

Although fairly stable resistance was demonstrated in the F_9 generation, subsequent attempts to reproduce these findings in the F_{22} and F_{24} generations were unsuccessful notwithstanding further increases in resistance from 9.46-fold in the F_9 to 13.4-fold in the F_{24}. In both cases there was a drastic loss of resistance within one to four generations almost to the level of the original parental strain. No definitive explanation can be offered at this stage for the subsequent instability of resistance. It is likely that under field conditions, where there is a broad exchange of genetic material between selected and unselected populations, such instability of resistance may not manifest itself.[1]

Nature of resistance in strain L. Although in the great majority of cases resistance has been traced to physiological mechanisms responsible for the degra-

[1] See Table 3, footnote *b*.

FIG. 2
RELATIVE DEGREE OF METABOLISM OF ^{14}C-LABELLED BAYGON BY LARVAE OF SUSCEPTIBLE (COACHELLA) AND RESISTANT (BAYGON L) STRAINS OF *C. FATIGANS* [a]



[a] Rf = distance solute moves ÷ distance solvent moves.

dation of the chemical, a few instances exist in which reduced rates of penetration of the insecticide were responsible for moderate increases in tolerance. In studies of malathion-resistance in *Aedes aegypti*, Matsumura & Brown (1961, 1963) found that a 5-fold tolerance to this insecticide and a low level of cross-tolerance to DDT were associated with decreased absorption of these insecticides by larvae.

The presence of increased ability to metabolize Baygon was demonstrated in our strain L in a limited number of tests by the use of ^{14}C-labelled Baygon. Groups of 30 fourth-instar larvae of strains L and Coachella were exposed for two hours to a concentration of 10 ppm of the labelled carbamate. They were afterwards rinsed with acetone and homogenized in 0.5 ml acetonitrile to which a few crystals of sodium sulfide were added. The homogenate was centrifuged for 5 minutes at 4000 rpm and the supernatant was chromatographed on propylene-glycol-impregnated paper strips. The latter were tested for radioactivity on an automatic scanning device (Vanguard Autoscanner 880). Fig. 2 indicates that the relative proportion of metabolites recorded was 12.4% in the Coachella strain and 30.4% in strain L. This amounts to an increase of 2.5-fold in carbamate degradation efficiency in strain L.

While providing evidence for physiological resistance to Baygon in strain L, these results do not exclude the presence of additional mechanisms of resistance, such as a reduced rate of penetration of the insecticide to the site of action. Details of further work on the characterization of resistance will be published in a subsequent paper.

Development of resistance by adult pressure in strain A

Selection of strain A by Baygon pressure, applied to the adult stage only, resulted in higher adult resistance than that observed in adults of strain L. Table 4 indicates an increase in tolerance amounting to 8.4-fold and 5.3-fold as determined by contact and topical application, respectively. The ld-p line for strain A, established by contact exposure to treated glass-paper (Fig. 3) failed to rise above the 90% mortality level even when the concentration of the residue was increased 100-fold. This characteristic of resistance in strain A was observed as early as the F_{18} generation and has persisted almost unchanged since then.

Interestingly, the relatively high adult resistance in strain A was not manifested in the larval stage, the LC_{50} for larvae of F_{24} being 0.625 ppm, i.e., only 2.7-fold higher than in the Laboratory strain (Table 2).

The plateau in the ld-p line of strain A, obtained by contact exposure, raises the possibility of behaviouristic avoidance of the residue in approximately 10% of the population of this strain under the conditions of the testing procedure. This might have resulted from gradual selection in favour of individuals that exhibited greater phototropism by

TABLE 4

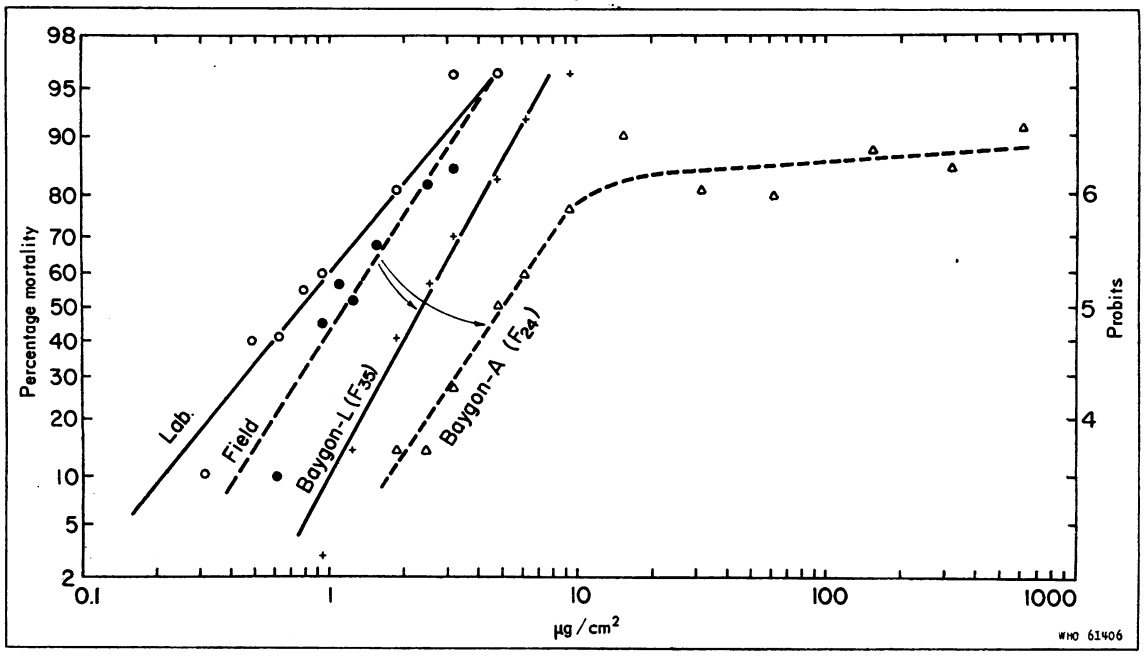
SUSCEPTIBILITY TO BAYGON IN ADULTS OF BAYGON-SELECTED AND NON-SELECTED STRAINS OF *C. FATIGANS*, AS DETERMINED BY CONTACT AND TOPICAL APPLICATION

Strain	Generation	LC_{50} or LD_{50}	95 % Fiducial limits	Slope (*b*) ± SE (*b*)	RR [a]
			Contact toxicity [b]		
Laboratory		0.785	0.561-0.999	2.250±0.260	1.0
Coachella		1.171	0.930-1.422	2.877±0.442	1.5
Baygon L	F_{35}	2.393	2.223-2.576	3.418±0.219	3.0
Baygon A	F_{24}	6.699	1.714-15.711	0.916±0.215	8.5
			Topical application [c]		
Laboratory		0.0069	0.0056-0.0084	1.369±0.109	1.0
Coachella		0.0126	0.0104-0.0152	2.445±0.329	1.8
Baygon L	F_{35}	0.0331	0.0279-0.0392	1.621±0.129	4.8
Baygon A	F_{24}	0.0368	0.0319-0.0424	2.189±0.207	5.3

[a] RR (resistance ratio) = LC_{50} resistant strain ÷ LC_{50} Laboratory strain.
[b] µg/cm².
[c] µg/insect.

FIG. 3

LD-P LINES FOR CONTACT TOXICITY OF BAYGON TO VARIOUS BAYGON-SELECTED AND NON-SELECTED STRAINS OF *C. FATIGANS*



tending to rest on the cloth cover of the vials, thus avoiding contact with the filter-paper. This point was tested by confining mosquitos of the various strains for two hours in glass vials (2.1 cm × 8.4 cm) lined with untreated filter-paper; at definite intervals those resting on the covers were counted. After each count the vials were disturbed in order to induce relocation of the mosquitos. The test was performed twice, first 20 and then 5 mosquitos per vial being used. The counts for each strain have been averaged and are presented, with their standard deviations, in Table 5. It is evident that strain A showed lower phototropic tendencies than strain L or the two unselected strains. Thus, phototropism cannot account for the high survival of strain A in the contact toxicity tests. A likely explanation would be an enhanced rate of metabolism of the insecticide in adults of this strain, the effects of which were probably magnified by reduction in tarsal absorption of the toxicant.

TABLE 5

PHOTOTROPISM IN ADULTS OF BAYGON-SELECTED AND NON-SELECTED STRAINS OF *C. FATIGANS* EVALUATED IN GLASS VIALS LINED WITH FILTER-PAPER

Strain	No. of mosquitos used	Percentage attracted to light [a]
	Test I [b]	
Coachella	80	19.67 ± 1.44 *, **
Baygon L	60	13.11 ± 1.12 **
Baygon A	160	15.78 ± 0.91 *
	Test II [c]	
Laboratory	100	12.45 ± 0.32 †
Coachella	100	16.36 ± 2.66 *, ††
Baygon L	100	10.45 ± 0.76 *, **
Baygon A	100	6.64 ± 0.72 **, †, ††

[a] Any two numbers bearing the superscripts * in each test are significantly different at the 5% level; those with superscripts **, †, and †† are significantly different at the 1% level.

[b] Average of nine 5-min readings; 20 insects/vial.

[c] Average of eleven 15-min readings; 5 insects/vial.

Cross-resistance

A comparison of the susceptibility levels of the Laboratory, Coachella, and Baygon L (F_{35}) strains to various insecticides is given in Table 6. The parental Coachella strain was slightly more tolerant than the Laboratory strain to most organophosphorus and carbamate insecticides, the tolerance in one case being as high as 3.9-fold (compound XI), while in other cases it was significantly below the level of the Laboratory strain (compounds II, VII, IX, XII, XIII, XVII, XXV). The Coachella strain contained, in addition, DDT-resistant individuals and also exhibited a substantial degree of tolerance toward dieldrin (Fig. 4). It is probable that the presence of DDT-resistance, and of generalized tolerance towards various other compounds, may have influenced both the rate of development of resistance to Baygon and the type of multiple resistance exhibited by the selected strain. For if resistance to carbamates requires the contribution of a number of ancillary genes, as is the case with other types of resistance, then the Coachella strain, having been selected in the field by exposure to DDT, cyclodiene, and to a lesser extent by organophosphate pressure, was already in a position to respond more quickly to further insecticidal selection, assuming, of course, that the various types of resistance in question share in common some of these ancillary genes. For the same reason, any multiple resistance spectrum resulting from carbamate selection in strain L must be interpreted in the light of the initial tolerance spectrum of the parental strain at the time of initiation of such selection.

Since it is known that the parental Coachella strain had not been exposed to carbamate pressure prior to its colonization, it may be assumed that the observed spectrum of cross-resistance to carbamates, following Baygon pressure, reflects the specific action of any major or minor genes selected by this process and therefore represents cross-resistance *per se*. On the other hand, the intensification of an existing resistance, such as that to DDT, may have been the result of chance selection due to close linkage between DDT- and Baygon-resistance genes. It may also have been the consequence of a minor gene in the DDT-resistant genotype imparting a selective advantage to this genotype in the carbamate environment. Thus, major DDT-resistance genes may be selected concurrently although they may not be directly involved in carbamate-resistance. Such cases should most appropriately be referred to as multiple resistance (Milani, 1963; Georghiou, 1965a). The following data are discussed in this context.

Carbamates. The data in Table 6 indicate that strain L, in addition to exhibiting 25.4-fold resistance to Baygon (III), also shows varying levels of cross-resistance to all other carbamates tested. The levels

TABLE 6

TOXICITY OF VARIOUS INSECTICIDES TO LARVAE OF TWO SUSCEPTIBLE (LABORATORY, COACHELLA) STRAINS AND A BAYGON-SELECTED (BAYGON L) STRAIN OF *C. FATIGANS*

Compound No.	Laboratory strain	Coachella strain		Baygon L strain	
	LC_{50} in ppm (and 95 % fiducial limits)	LC_{50} in ppm (and 95 % fiducial limits)	RR [a]	LC_{50} in ppm (and 95 % fiducial limits)	RR [a]
I	0.412 (0.388-0.438)	0.753 (0.667-0.848)	1.8	8.272 (7.954-8.603)	20.1
II	0.0355 (0.0298-0.041)	0.0281 (0.0253-0.0311)	0.8	0.312 (0.299-0.325)	8.8
III	0.228 (0.219-0.237)	0.257 (0.236-0.278)	1.1	5.785 (5.580-5.997)	25.4
IV	0.764 (0.742-0.786)	1.150 (1.098-1.204)	1.5	6.537 (6.305-6.779)	8.6
V	1.17	1.625 (1.519-1.738)	1.4	4.009 (3.902-4.118)	3.4
VI	0.275 (0.261-0.289)	0.257 (0.247-0.267)	0.9	2.928 (2.469-3.526)	10.6
VII	0.0337 (0.0321-0.0353)	0.0292 (0.0281-0.0305)	0.9	0.206 (0.179-0.235)	6.1
VIII	0.877 (0.797-1.002)			>10.0	>10.0
IX	1.5	0.910	0.6	5.300 (4.986-5.634)	3.5
X	0.104 (0.0965-0.111)	0.154 (0.148-0.160)	1.5	1.170 (1.120-1.223)	11.3
XI	0.046	0.181 (0.067-0.284)	3.9	0.304 (0.293-0.315)	6.6
XII	0.284 (0.249-0.343)	0.181 (0.166-0.197)	0.6	2.225 (2.095-2.363)	7.8
XIII	0.623 (0.597-0.651)	0.471 (0.423-0.513)	0.8	6.453 (6.154-6.767)	10.4
XIV	0.0524 (0.0508-0.054)	0.048 (0.0446-0.0517)	0.9	1.397 (0.756-1.996)	26.7
XV	0.0716 (0.0693-0.074)	0.0653 (0.0627-0.0681)	0.9	0.603 (0.580-0.627)	8.4
XVI	0.104 (0.0963-0.111)	0.262 (0.232-0.303)	2.5	0.503 (0.471-0.537)	4.8
XVII	1.2	0.890 (0.804-0.957)	0.7	5.563 (5.212-5.938)	4.6
XVIII	0.300 (0.289-0.311)	0.343 (0.272-0.441)	1.1	3.294 (3.150-3.445)	11.0
XIX	0.127 (0.118-0.137)	0.21	1.7	0.933 (0.890-0.977)	7.3
XX	0.423 (0.404-0.444)	0.652 (0.622-0.684)	1.5	3.287 (3.153-3.427)	7.8
XXI	0.595 (0.552-0.643)	1.410 (1.005-1.942)	2.4	4.344 (4.136-4.564)	7.3
XXII	0.055 (0.0535-0.0565)	0.056 (0.053-0.058)	1.0	0.375 (0.356-0.396)	6.8
XXIII	0.765 (0.700-0.835)	1.676 (1.552-1.810)	2.2	10.773 (10.379-11.182)	14.1
XXIV	0.513 (0.461-0.566)	0.856 (0.680-1.052)	1.7	1.084 (1.002-1.174)	2.1
XXV	0.877 (0.797-1.002)	0.618 (0.585-0.652)	0.7	3.171 (2.890-3.479)	3.6
XXVI	0.156 (0.153-0.159)	0.161 (0.155-0.167)	1.0	0.342 (0.332-0.352)	2.2
Dieldrin	0.00755 (0.00711-0.008)	0.00829 (0.00731-0.00939)	1.1	0.0779 (0.0751-0.0808)	10.3
DDT	0.0255 (0.0243-0.0267)	0.109 (0.0975-0.122)	4.3	1.701 (1.271-2.219)	66.7
Malathion	0.0813 (0.0789-0.0837)	0.124 (0.117-0.131)	1.5	0.671 (0.654-0.688)	8.3
Fenthion	0.0077	0.0091 (0.0086-0.0096)	1.2	0.051	6.6
Fenitrothion	0.0222 (0.0203-0.0248)	0.0197 (0.0178-0.0222)	0.9	0.0395 (0.0361-0.0429)	1.8
Dursban	0.0022			0.0024	1.1

[a] RR (resistance ratio) = LC_{50} strain studied ÷ LC_{50} Laboratory strain.

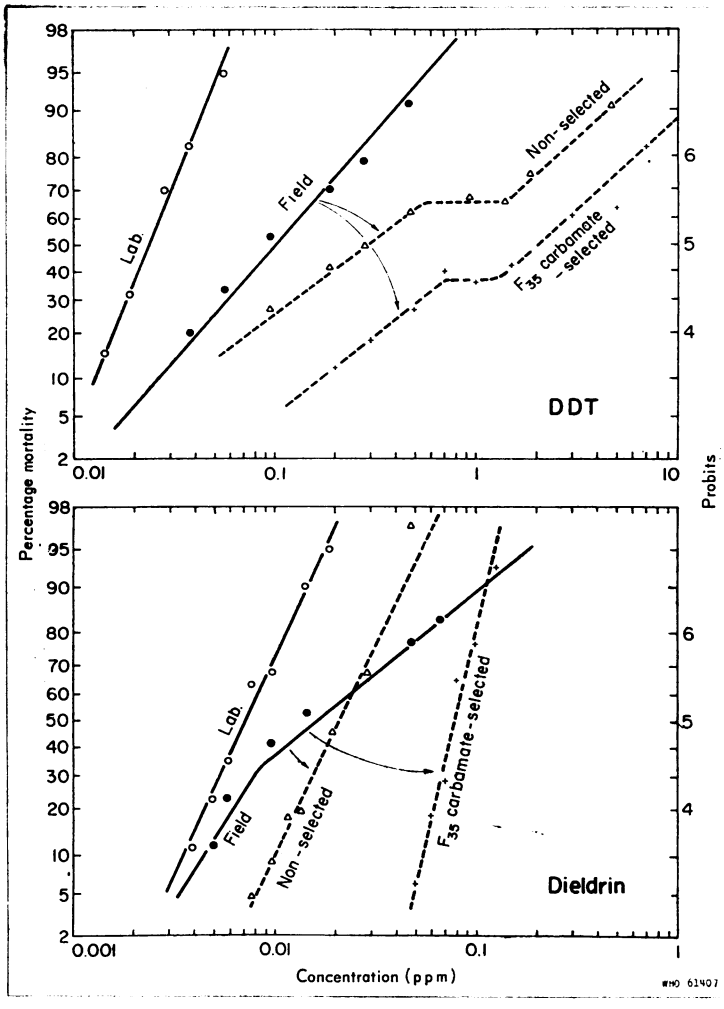


FIG. 4
RELATIVE SUSCEPTIBILITY TO DDT AND DIELDRIN IN BAYGON-SELECTED AND NON-SELECTED STRAINS OF *C. FATIGANS* [a]

[a] Field = Coachella strain.

of such cross-resistance reflect to a certain extent the structural relationships between these compounds and the selective agent. Thus, cross-resistance to the closely related compound I (*o*-isopropylphenyl methylcarbamate) and compound XIV (2,3-dihydro-2,2-dimethyl benzofuranyl-7-methylcarbamate) is very high, i.e., 20.1-fold and 26.7-fold, respectively, while towards the remotely related thiolane compounds and Temik (XXIV-XXVI) cross-resistance is the lowest (2.1-fold to 3.6-fold). Intermediate cross-resistance (4.6-fold to 11.0-fold) was present toward various di- and tri-substituted phenyl methylcarbamates (compounds XV-XXI). Cross-resistance to the xylyl methylcarbamates (XIX-XXI) is strikingly similar (7.3-fold to 7.8-fold) despite the differences in the intrinsic toxicity of these compounds.

A certain degree of specificity is evident towards the *ortho*-substituted phenyl methylcarbamates in agreement with the position of the $-OC_3H_7$ substituent on the phenyl ring of Baygon. Thus, resistance to compound I (*ortho*) is 20.1-fold while to the *meta*-substituted analogue (II) it is only 8.8-fold. The same relationship is also true in the other pairs of *ortho*- and *meta*-substituted compounds studied, i.e., IV-V, VI-VII, and VIII-IX.

Although these cross-resistance data were determined on the F_{35} generation of strain L, cross-resistance to carbamates appeared early during selection and its extent increased progressively, at a

rate roughly commensurate with the increase in resistance to Baygon. It thus represents an expression of the activity spectrum of the various genes involved in Baygon-resistance.

The information obtained on cross-resistance indicates that selection of *C. p. fatigans* by Baygon results in the development of a mechanism of carbamate-resistance specific for Baygon and for a small number of closely related materials. This mechanism extends also to carbaryl (XXIII) and, to a smaller extent, to various other substituted phenyl methylcarbamates. Of interest, also, is the low level of cross-resistance (3.4-fold) to *m*-propargyloxyphenyl methylcarbamate (V), a situation observed, as well, in houseflies exhibiting high levels of resistance to *m*-isopropylphenyl methylcarbamate (II).[1]

Organophosphates. The levels of cross-resistance to four organophosphates (Table 6) varied from very low (1.1-fold) to Dursban to moderate (8.3-fold) to malathion. In view of the structural differences among these compounds, and of the different enzymes believed to be responsible for their degradation, it could hardly be expected that equal cross-resistance levels would be present to these compounds. The observed cross-resistance may be explained, in part, in terms of enhancement of pre-existing tolerances, since the parental strain had been subjected to organophosphate treatments prior to its colonization. It may also be partly due to factors involved in organophosphate-resistance as well as in carbamate-resistance, in view of the relationship between these two classes of compounds as cholinesterase inhibitors. Thus, characterization of the observed resistance as multiple- or cross-resistance must await further elucidation of the biochemical and genetic mechanisms involved in such resistance.

DDT. The results of treatment with DDT (Fig. 4, upper graph) indicate that the parental strain (Coachella) contained a certain proportion of DDT-resistance genes at the time of initiation (1963) of selection by Baygon. This is evident in the considerably lower slope of the ld-p line as compared with that of the Laboratory strain ($b=2.18$ *versus* 4.87), and also in the 4.3-fold higher LC_{50} value (Table 6). This pre-existing DDT-resistance increased progressively during selection with Baygon so that by the F_{35} generation it attained a level 66.7-fold higher than that of the Laboratory strain. A definite plateau is evident in the ld-p line. It must be pointed out, however, that the unselected Coachella strain also gained in DDT-resistance, possibly as a result of the laboratory manipulation of the strain, although such increase was below that observed in the Bayon-selected strain.

Dieldrin. The slope of the ld-p line of the parental field strain (Coachella) (Fig. 4, lower graph) was considerably lower than that of the Laboratory strain, indicating the presence of at least incipient resistance to dieldrin. Selection of the strain by Baygon caused a steepening of the regression line, so that by the F_{35} generation a 10.3-fold increase in the LC_{50} had occurred, i.e., to 0.078 ppm. This is lower than the LC_{50} level characteristic of homozygous-resistant populations, i.e., 0.45 ppm (Pennell & Hoskins, 1964). It must be noted, also, that the regression line of the Baygon-selected strain did not extend beyond the upper limits of the susceptibility range of the parental population, i.e., 0.2 ppm. It was thus suspected that the observed increase in tolerance to dieldrin was a case of " vigour tolerance " rather than a specific dieldrin-resistance. This hypothesis was tested further by subjecting a substrain of the Coachella population to selection pressure by dieldrin in the larval stage (Fig. 5). After 15 generations of rigorous selection, the level of response to dieldrin was identical to that observed in the Baygon-selected strain. If any specific gene for dieldrin-resistance was present in the Coachella strain, its frequency would almost certainly have been increased, and high dieldrin-resistance would have developed, within 15 generations of selection. Thus, the identical increase in tolerance to dieldrin obtained by Baygon or dieldrin selection pressure may be considered a case of vigour tolerance as defined by Hoskins & Gordon (1956).

Synergism of carbamates against mosquitos

The remarkable synergism of carbamates by methylene dioxyphenyl derivatives has been studied by several investigators (Moorfield, 1958; Eldefrawi et al., 1959, 1960; Fuchs & Zschintzsch, 1959; Metcalf et al., 1960; Georghiou et al., 1961; Fukuto et al., 1962; Georghiou, 1962; Moorfield & Weiden, 1964; Wilkinson et al., 1966). Certain other compounds, such as 2-(3,5dichloro-2-biphenylyloxy)-triethylamine (Moorfield & Tefft, 1959), octachlorodipropylether (Georghiou & Metcalf, 1961a) and organothiocyanates (El-Sebae et al., 1964) have also shown synergistic activity on carbamates.

[1] G. P. Georghiou, unpublished data.

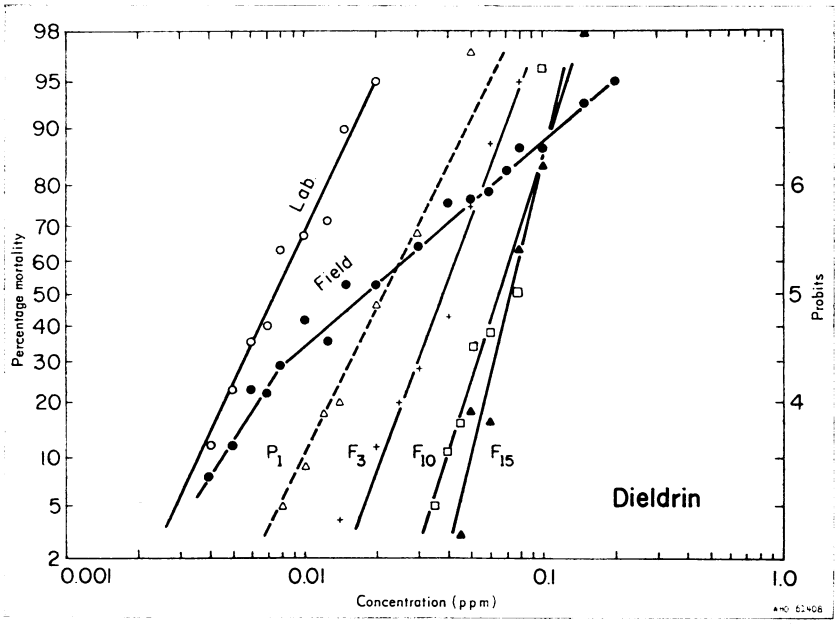


FIG. 5
RESULTS OF LARVAL SELECTION OF *C. FATIGANS* WITH DIELDRIN FOR 15 GENERATIONS

These studies offer an insight into the mechanism of carbamate metabolism and resistance, and are also of significance in the practical application of the principle of synergism in insect control. While most of the basic studies of carbamate synergism have been carried out on houseflies, the phenomenon of carbamate synergism is rather general in insects, as demonstrated in the bean aphid, *Aphis fabae* Scop. (Moorfield, 1958), the German cockroach, *Blatella germanica* L. (Moorfield, 1958), the vinegar fly, *Drosophila melanogaster* Meigen (Fuchs & Zschintzsch, 1959), the body louse, *Pediculus humanus humanus* L. (Cole & Clark, 1962), the honeybee, *Apis mellifera* L. (Georghiou & Atkins, 1964), the grasshopper, *Melanoplus bilituratus* Wlk. (McKinlay, 1965), and others.

Georghiou & Metcalf (1961b) investigated the synergistic action of carbamate–piperonyl-butoxide combinations on mosquitos and found only limited potentiation against the larvae of the Laboratory strain of *C. p. fatigans*. The synergism ratios observed in these studies ranged from 1.5-fold for the more active carbamate (II) to 7-fold for the least active (Isolan). The same phenomenon was also true in adult mosquitos, the degree of synergism being, in general, inversely proportional to the toxicity of the carbamate. This limited degree of synergism was thought to be due to absence of a strong mechanism for carbamate degradation in the strain studied. The subsequent development of relatively high resistance to Baygon in *C. p. fatigans* has presented the opportunity for additional studies on the synergism of carbamates in this species.

Tests were carried out with piperonyl butoxide and other synergists against larvae, and to a limited extent against adults of the parental Coachella strain, as well as the Baygon-selected strains. The results with the Coachella strain (Table 7) indicate limited synergism of carbamates, in agreement with the earlier results obtained on the Laboratory strain (Georghiou & Metcalf, 1961b). Tests on strain Baygon L (F_{10}) also produced a relatively low degree of synergism, ranging from 1.2-fold for compound XXIV to 5.2-fold for compound XXI. Synergism of Baygon was 4-fold although the Baygon-resistance level of the strain was then 8.8-fold. Repetition of these tests on larvae of the F_{35} generation gave a synergism ratio for Baygon of 5.4-fold while the resistance level of the strain was then 25.4-fold higher than originally. It is thus obvious that the highest degree of synergism obtained by piperonyl butoxide against larvae is considerably lower than the degree of resistance to that compound, a situation notably different from that observed in houseflies, in which this synergist virtually restores the intrinsic toxicity of the carbamate against the

TABLE 7
SYNERGISM OF CARBAMATES BY VARIOUS COMPOUNDS AGAINST *C. FATIGANS*

Strain	Stage	Carbamate	Synergist	LC_{50} [a]		Synergism ratio (A/B)
				Carbamate alone (A)	Carbamate+synergist [b] (B)	
Coachella	Larvae	Baygon	Piperonyl butoxide [c]	0.29 (0.28-0.31)	0.13 (0.12-0.15)	2.2
		Baygon	Sesoxane [d]		0.27 (0.25-0.28)	1.1
		V	Piperonyl butoxide [c]	1.63 (1.52-1.74)	0.74 (0.70-0.77)	2.2
		XXIV	Piperonyl butoxide [c]	0.86 (0.68-1.10)	0.84 (0.61-1.25)	1.0
Baygon L (F_{10})	Larvae	Baygon	Piperonyl butoxide	2.00 (1.95-2.05)	0.56 (0.53-0.58)	3.6
		Baygon	Sesoxane		1.10 (1.04-1.17)	1.8
		V	Piperonyl butoxide	1.84 (1.79-1.89)	0.90 (0.86-0.94)	2.0
		X	Piperonyl butoxide	0.67 (0.62-0.72)	0.26 (0.21-0.31)	2.6
		XX	Piperonyl butoxide	2.74 (2.54-2.94)	1.01 (0.79-1.31)	2.7
		XXI	Piperonyl butoxide	3.41 (2.83-4.44)	0.66	5.2
		XXIV	Piperonyl butoxide	1.31 (1.21-1.41)	1.08 (0.99-1.17)	1.2
Baygon L (F_{35})	Larvae	Baygon	Piperonyl butoxide	5.79 (5.58-6.00)	1.08 (1.06-1.10)	5.4
		Baygon	Thanite [e]		1.63 (1.58-1.68)	3.6
		Baygon	TOCP [f]		3.09 (2.97-3.22)	1.9
Baygon A (F_{25})	Adults	Baygon	Piperonyl butoxide [c]	6.70 (1.71-15.71)	1.49 (1.25-1.74)	4.5

[a] LC_{50} in ppm for larvae, in $\mu g/cm^2$ for adults.
[b] 1 : 5 ratio (except Baygon: TOCP = 1 : 10).
[c] LC_{50} for piperonyl butoxide alone = 23.5 ppm.
[d] LC_{50} for Sesoxane alone >10 ppm.
[e] LC_{50} for Thanite alone 22 ppm.
[f] LC_{50} for TOCP (tri-*o*-cresylphosphate) alone >100 ppm.

resistant strain. In contrast to the better performance of Sesoxane than of piperonyl butoxide as a carbamate synergist in houseflies (Eldefrawi et al., 1959), Sesoxane was inferior to piperonyl butoxide as a synergist of Baygon in mosquito larvae.

Other carbamate synergists such as Thanite (El-Sebae et al., 1964) and tri-*o*-cresylphosphate (TOCP) (Metcalf & Fukuto, 1965) also produced rather low synergism ratios with Baygon against strain L (Table 7).

The synergistic activity of piperonyl butoxide was also investigated against adult mosquitos of strain A (F_{25}), in view of the high resistance to Baygon in this stage as determined by the contact method of exposure (Fig. 3). Piperonyl butoxide was applied to glass filter-paper together with Baygon at the ratio of 5 : 1. Under these conditions the presence of piperonyl butoxide considerably enhanced the toxicity of Baygon. A straight ld-p line was obtained ($b = 3.294 \pm 0.355$) with an LC_{50} of 1.488, i.e., only 1.3-fold and 2-fold greater than the LC_{50}s for synergized Baygon against the Coachella and Laboratory strains, respectively. These data indicate strong synergism of Baygon against adult mosquitos, the degree of such synergism approaching that observed in houseflies. The contribution of the physical presence of an oily film of piperonyl butoxide in the uptake of Baygon by mosquitos is not known. However, tests with Baygon in combination with ether–Risella-oil on glass filter-paper, as in the WHO test method, produced LC_{50} values higher than those obtained with Baygon and piperonyl butoxide, thus leaving little doubt as to the strong synergistic effect of the latter.

An interesting case of synergism of carbamates is presented by 2,3-naphthalenediol, methylene ether (Table 8). Significant synergism of Baygon was obtained in larvae, at ratios of synergist as low as

TABLE 8

SYNERGISM OF BAYGON BY 2,3-NAPHTHALENEDIOL, METHYLENE ETHER [a] AGAINST LARVAE OF *C. FATIGANS*

Strain	Baygon: synergist ratio	LC_{50} in ppm (and 95% fiducial limits)	Synergism ratio [b]
Laboratory	Synergist alone	6.53 (6.35-6.72)	—
	Baygon alone	0.29 (0.27-0.31)	—
	1 : 5	0.20 (0.19-0.21)	1.45
	1 : 1	0.23 (0.22-0.25)	1.26
	1 : 0.1	0.25 (0.23-0.28)	1.16
Baygon L (F_{11})	Synergist alone	7.00	—
	Baygon alone	1.84 (1.78-1.91)	—
	1 : 5	0.46 (0.44-0.47)	4.00
	1 : 1	0.87 (0.84-0.91)	2.11
	1 : 0.1	1.50 (1.35-1.74)	1.23
	1 : 0.01	1.83 (1.73-1.93)	1.01

[a] Fundamental Research Co., Berkeley, Calif.
[b] LC_{50} carbamate alone ÷ LC_{50} carbamate + synergist.

0.1 to 1 of carbamate. In this case, however, the synergist by itself is also toxic to larvae at concentrations greater than 5 ppm (LC_{50}=7.0 ppm).

GENERAL CONSIDERATIONS

The primary objective of laboratory selections for resistance is to obtain information on the extent and rate of attainable resistance. These are important considerations in any decision to proceed with the further development of a certain compound, or to adopt it in any large-scale or long-term pest control operation.

On the basis of past experience in chemical control, there remains little doubt that, given adequate selection pressure in the field, some resistance will eventually appear. Thus, the crucial question is not whether resistance will occur, but how soon, at what rate, and to what extent.

It may be well to point out that negative results in selection for resistance in the laboratory may be due to accidental exclusion of suitable genetic material from the initial population or from subsequent generations, rather than to absence of resistance potential for a particular insecticide in the species as a whole. In fact, laboratory selections of mosquitos for resistance have more often given negative than positive results, although field populations eventually did develop resistance. Thus, selection of *C. p. fatigans* with malathion in our laboratory for 50 generations failed to produce resistance (Georghiou, 1966), although such resistance appeared in field populations of this species. Many other examples could be cited. Positive results, however, increase the probability of development of resistance in the field, so that field operations can then be geared with that expectation in prospect.

The results of larval selection of *C. p. fatigans* with Baygon reported here (strain L) indicate that despite a marked (4-fold) increase in tolerance in the F_1 generation, further progress in the development of resistance was slow, attaining a 25.4-fold level in 35 generations of rigorous selection at the 80%-95% mortality level. These results suggest a very slow grouping of the genetic material which contributes to resistance and thus the likelihood of involvement of several chromosomal segments in such resistance. Of interest in this connexion are the steep slope values of the ld-p lines of the several generations tested throughout the progress of selection, a condition suggesting the lack of an "all-powerful" resistance gene that produces several-fold resistance the presence or absence of which in a fraction of the population would increase the variance of response to the toxicant. Resistance to Baygon in this strain was reinforced gradually and in small doses, so that no abrupt break in the ld-p line occured. This trend is in contrast to the selection for resistance towards chlorinated hydrocarbons and dieldrin, which is typified by low slopes in the ld-p lines.

The cross-resistance spectrum of the Baygon selected strain indicates that resistance consists o one or several broad defence mechanisms effective on a wide variety of carbamates and organophosphates, plus a more discrete mechanism, specific for Baygon and for other closely related carbamates. In this respect the results are encouraging as they indicate that the eventual development of resistance to Baygon does not preclude the use of other carbamates against the resistant population.

The low degree of expressivity of resistance in adults of the larval-selected strain (L), and similarly in larvae of the adult-selected strain (A), is of interest from the practical aspect of mosquito control, and also in furthering an understanding of the nature of resistance. This resistance phenomenon tends to suggest that in addition to the presence of a mechanism common to both stages—larval and adult—other resistance mechanisms also exist which are limited to each specific life stage against which the selective pressure is directed.

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RÉSUMÉ

Les auteurs ont étudié la rapidité d'apparition et les modalités de la résistance au Baygon dans une colonie récemment établie de *Culex pipiens fatigans* originaire de la Californie. La sélection des larves par des doses d'insecticide provoquant une mortalité de 80%-95% a été poursuivie pendant 35 générations (souche L). La résistance chez les larves a atteint le niveau 4 fois à la génération F_1, puis a augmenté très progressivement pour atteindre le niveau 25,4 fois à la génération F_{35}. Au cours des tests, les courbes dose-mortalité ont montré des valeurs de pente élevées chez toutes les générations sélectionnées, suggérant l'absence d'un gène « tout-puissant » de résistance aux carbamates et contrastant avec les caractéristiques de la résistance aux hydrocarbures chlorés. L'augmentation de la tolérance chez les adultes de cette souche n'a été que de 3 fois et 4,8 fois respectivement, suivant que l'on a utilisé les méthodes de contact ou d'application locale de l'insecticide.

Réciproquement, une souche sœur sélectionnée au stade adulte par action du Baygon (souche A) a montré chez l'adulte une résistance de 8,4 fois (contact) et de 5,3 fois (application locale) et seulement une résistance de 2,7 fois chez les larves. Ainsi, en dehors d'un mécanisme de développement de la résistance commun aux deux stades, il existe aussi d'autres mécanismes particuliers au stade évolutif, larve ou imago, contre lequel la pression sélective est exercée.

Environ 10% des adultes de la souche A ont été capables de survivre après un contact avec l'insecticide, même lorsque la concentration de Baygon a été augmentée 100 fois. Cette survie ne doit pas être attribuée à une tendance accrue à éviter le contact avec le papier filtre placé dans la chambre d'exposition. En utilisant le Baygon marqué au ^{14}C, on a pu montrer que les larves de la souche L métabolisaient le carbamate 2,5 fois plus vite que les larves de la souche non sélectionnée.

La résistance au Baygon des larves de la souche L s'est accompagnée d'une forte résistance croisée aux carbamates étroitement apparentés, comme l'OMS 32 et l'OMS 864. La résistance croisée a été moindre à l'égard de divers phénylméthylcarbamates di- et trisubstitués et moindre encore envers des insecticides de formule moins voisine.

La résistance croisée aux organo-phosphorés a varié de 1,8 fois pour le fenitrothion à 8,3 fois pour le malathion. Chez la souche L, on a noté un accroissement de la résistance préexistante au DDT mais la modification de la sensibilité à la dieldrine ne s'est manifestée que par une augmentation de la pente de la courbe dose-mortalité qui a été considérée comme résultant d'une tolérance de vigueur.

Les auteurs concluent que le développement relativement lent de la résistance au Baygon chez *C. pipiens fatigans*, la faible expression de ce caractère chez les adultes et sa spécificité vis-à-vis de l'insecticide utilisé pour la sélection et les carbamates étroitement apparentés permettent d'envisager favorablement l'emploi de ce groupe de produits dans la lutte contre les moustiques.

REFERENCES

Abedi, Z. H. & Brown, A. W. A. (1960) *Canad. J. Genet. Cytol.*, **2**, 252

Bar-Zeev, M. & Bracha, P. (1965) *Bull. Wld Hlth Org.*, **33**, 461

Bull. Wld Hlth Org., 1963, **29**, Suppl.

Brown, A. W. A., Lewallen, L. L. & Gillies, P. A. (1963) *Mosquito News*, **23**, 341

Cole, M. M. & Clark, P. H. (1962) *J. econ. Ent.*, **55**, 98

Eldefrawi, M. E., Miskus, R. & Hoskins, W. M. (1959) *Science*, **129**, 898

Eldefrawi, M. E., Miskus, R. & Sutcher, V. (1960) *J. econ. Ent.*, **53**, 231

El-Sebae, A. H., Metcalf, R. L. & Fukuto, T. R. (1964) *J. econ. Ent.*, **57**, 478

Finney, D. J. (1952) *Probit analysis*, 2nd ed., London, Cambridge University Press

Fox, I. & Garcia-Moll, I. (1961) *Science*, **133**, 646

Fuchs, W. H. & Zschintzsch, J. (1959) *Naturwissenschaften*, **46**, 273

Fukuto, T. R., Metcalf, R. L., Winton, M. Y. & Roberts, P. A. (1962) *J. econ. Ent.*, **55**, 341

Gahan, J. B., LaBrecque, G. C. & Wilson, H. G. (1961a) *J. econ. Ent.*, **54**, 63

Gahan, J. B., LaBrecque, G. C. & Wilson, H. G. (1961b) *Mosquito News*, **21**, 289

Gahan, J. B., LaBrecque, G. C. & Wilson, H. G. (1962) *Mosquito News*, **22**, 333

Gahan, J. B., Wilson, H. G. & Smith, C. N. (1964) *Bull. Wld Hlth Org.*, **30**, 139

Georghiou, G. P. (1962) *J. econ. Ent.*, **55**, 768

Georghiou, G. P. (1963) *Effects of carbamate insecticide selection pressure on* Anopheles albimanus *Wied.* In: California Mosquito Control Association, *Proceedings and papers of the Thirty-first Annual Conference of the California Mosquito Control Association*, Visalia, Calif., p. 80

Georghiou, G. P. (1965a) *Advanc. Pest Control Res.*, **6**, 171

Georghiou, G. P. (1965b) *Nature (Lond.)*, **207**, 883

Georghiou, G. P. (1965c) *Insecticide resistance with special reference to mosquitos.* In: California Mosquito Control Association, *Proceedings and papers of the Thirty-third Annual Conference of the California Mosquito Control Association*, Visalia, Calif., p. 34

Georghiou, G. P. (1966) *Research on mosquito resistance to insecticides at the University of California, Riverside.* In: California Mosquito Control Association, *Proceedings and papers of the Thirty-fourth Annual Conference of the California Mosquito Control Association*, Visalia, Calif. (in press)

Georghiou, G. P. & Atkins, E. L., Jr (1964) *J. apicult. Res.*, **3**, 31

Georghiou, G. P. & Gidden, F. E. (1965) *Mosquito News*, **25**, 204

Georghiou, G. P. & Metcalf, R. L. (1961a) *J. econ. Ent.*, **54**, 150

Georghiou, G. P. & Metcalf, R. L. (1961b) *Mosquito News*, **21**, 303

Georghiou, G. P. & Metcalf, R. L. (1961c) *Mosquito News*, **21**, 328

Georghiou, G. P. & Metcalf, R. L. (1963) *Science*, **140**, 301

Georghiou, G. P., Metcalf, R. L. & March, R. B. (1961) *J. econ. Ent.*, **54**, 132

Hamon, J. & Sales, S. (1963) *Méd. trop.*, **23**, 621

Hadaway, A. B. & Barlow, F. (1963) *Bull. Wld Hlth Org.*, **28**, 63

Hadaway, A. B. & Barlow, F. (1965a) *Bull. Wld Hlth Org.*, **32**, 581

Hadaway, A. B. & Barlow, F. (1965b) *Bull. Wld Hlth Org.*, **33**, 129

Hoskins, W. M. & Gordon, H. T. (1956) *Ann. Rev. Ent.*, **1**, 89

Kerr, J. A., deCamargo, S. & Abedi, Z. H. (1964) *Mosquito News*, **24**, 276

LaBrecque, G. C., Gahan, J. B. & Wilson, H. G. (1960) *Mosquito News*, **20**, 238

Lewallen, L. L. (1961) *Mosquito News*, **21**, 310

McKinlay, K. S. (1965) *Canad. Ent.*, **97**, 827

March, R. B. & Metcalf, R. L. (1949) *Bull. Calif. Dept. Agric.*, **38**, 93

Matsumura, F. & Brown, A. W. A. (1961) *Mosquito News*, **21**, 192

Matsumura, F. & Brown, A. W. A. (1963) *Mosquito News*, **23**, 26

Metcalf, R. L., Fuertes-Polo, C. & Fukuto, T. R. (1963) *J. econ. Ent.*, **56**, 862

Metcalf, R. L. & Fukuto, T. R. (1965) *J. agric. Food Chem.*, **13**, 220

Metcalf, R. L., Fukuto, T. R. & Winton, M. (1960) *J. econ. Ent.*, **53**, 828

Metcalf, R. L., Fukuto, T. R. & Winton, M. (1962) *J. econ. Ent.*, **55**, 889

Milani, R. (1963) *Bull. Wld Hlth Org.*, **29**, Suppl., p. 77

Moorfield, H. H. (1958) *Contrib. Boyce Thompson Inst.*, **19**, 501

Moorfield, H. H. & Tefft, E. R. (1959) *Contrib. Boyce Thompson Inst.*, **20**, 293

Moorfield, H. H. & Weiden, M. H. J. (1964) *Contrib. Boyce Thompson Inst.*, **22**, 425

Mulla, M. S. (1964) *Mosquito News*, **24**, 334

Pennell, J. T. & Hoskins, W. M. (1964) *Bull. Wld Hlth Org.*, **31**, 669

Schoof, H. F., Mathis, W., Brydon, H. W. & Goodin, W. J. (1964) *Amer. J. trop. Med. Hyg.*, **13**, 876

Vandekar, M. (1965) *Bull. Wld Hlth Org.*, **33**, 107

Wilkinson, C. M., Metcalf, R. L. & Fukuto, T. R. (1966) *J. agric. Food Chem.*, **14**, 73

WHO Expert Committee on Insecticides (1963) *Wld Hlth Org. techn. Rep. Ser.*, **265**